ON THE SULPHUR IN KERATINE

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Introduction.

The study of the proteins has always been of considerable importance, for these substances are of special interest in a consideration of the phenomena of life. Before the physiologist can solve the intricate problems of digestion and metabolism, the chemist must furnish the requisite knowledge concerning the true nature of the proteins. Proteins perform a variety of functions. From an anatomical point of view, proteins may be divided into two classes,- first, those that are immediately concerned in metabolism; and second, those proteins which are used for structural purposes. This second class was formerly called "albuminoids," but since Emil Fischer and others have found that these bodies consist of the same "building stones" of which all other proteins are built, the term "albuminoid" has been dropped, and the word "scleroproteins" has come into use. Sclero is derived from the Greek word meaning hard. All these harder and more resistant proteins have now been placed in one class, not because they are necessarily alike chemically, but because they fulfill similar anatomical functions and have similar resistant properties.

Cohnheim[1] divides the scleroproteins into the following groups:

Scleroproteins (Gerüsteiweisse)

1. Collagen.

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[1] Cohnheim - "Chemie der Eiweisskörper" pp. 177. (1911)

2. Keratine.

3. Fibroin.

4. Spongin.

5. Elastin.

6. Conchiolin.

7. Amyloid.

8. Ichthylepidin.

The literature concerning these scleroproteins is rather meagre; most of it is either very old or quite recent. It has been the aim of this research to gain a better knowledge of these scleroproteins, particularly of keratine.

The term keratine is derived from the Greek word meaning horn. Keratine is the main constituent of hoofs, horns, hair, feathers, and all epidermal tissues. Keratine is also found in the protective covering of the nerves. Tortoise shell consists of keratine and some forms of sea coral are composed of keratine in combination with iodine. The shell membrane of the egg also consists of keratine. The older researches on keratine did not reveal much concerning this protein. It was, however, found that keratine is extremely resistant to all chemical reagents, for it is attacked only by strong concentrated acids such as sulphuric and nitric acids. Hydrochloric acids attacks the material only upon long continued boiling. Dilute acids and alkalies have no action whatever in the cold. A hot, ten per cent caustic soda solution dissolves the keratine slowly. A twenty per cent caustic soda solution will dissolve the keratine slowly in the cold. In such a solution of keratine in caustic

soda, large amounts of sodium sulphide are formed. Now if lead acetate is added to the solution, a black precipitate of lead sulphide forms. This reaction has generally been described as characteristic of keratine, and is commonly called the lead blackening reaction. It has also been known that keratine gives a very decided xantho-protein reaction. This reaction results upon the addition of strong nitric acid to keratine. A light, yellow coloration of the sample is produced, which turns to a bright orange color if the acid is neutralized with an excess of ammonia.

The remainder of the earlier researches on keratine deal mainly with elementary analyses. The results of the earlier researches are tabulated below:

Reagent.	Effect upon Keratine.
Water	Absorbs a certain amount of water. Not soluble.
Steam under pressure	Decomposes with liberation of H_2S and methyl-mercaptan.
10% caustic soda (hot)	Undergoes only partial solution.
20% caustic soda.	Dissolves except small amount of residue. Large quantity of ammonia is developed and the solution contains a large amount of Na_2S.
Dilute acids.	Insoluble.
Concentrated sulphuric acid.	Soluble and undergoes hydrolysis.

Neutralization of solution of keratine in caustic soda.	Produces a resin-like precipitate.

E. Fischer and T. Dorpinghaus[1] in 1902 found the following amino acids as products of the hydrolysis of horn:

Acid.	Per cent.
Glycocoll	0.34
Alanine	1.20
Valine	5.7
Leucine	18.3
Asparagine	2.5
Glutamic acid	14.0
Serine	0.7
Proline	3.6
Phenylalanine	3.0
Tyrosine	4.58[2]
Arginine	2.25[3]

(1) Zeit für Physiol. Chemie 36, 462 (1902)

(2) E. Alderhalden and H. Voitinovici-Zeit. Physiol. Chemie, 52, 348 (1907)

(3) " " " H. G. Wells, -Zeit. Physiol. Chemie, 46, 31 (1905)

The elementary percentage composition of keratine from various sources as given by Hammersten, is as follows:

Source of Keratine	C	H	N	S	O
Human hair.	50.65	6.36	17.14	5.00	20.8
Finger Nails.	51.00	6.49	17.51	2.8	21.7
Neurokeratine.	56.11-58.45	8.02	11.4-14.3	1.6-2.20	--
Horn.	50.86	6.94	--	3.3	--
Tortoise shell.	54.89	6.56	16.7	2.2	19.56
Shell Membrane and egg.	49.78	6.64	16.4	4.2	22.9

The one notable feature of these analyses as compared with the analyses of other proteins, is the unusually high sulphur content. This characteristically high sulphur content of keratine was recognized quite early. It was also known that part of the sulphur in keratine is easily split off, whereas the remainder is firmly bound.

Some of the earlier investigators pointed out that lead combs which had been used for some time became covered with the black sulphide of lead, the sulphur coming from the hair. Then, too, the so-called lead blackening reaction was early recognized as characteristic of keratine; namely, if some keratine is heated with caustic alkali and lead acetate, a heavy precipitate of lead sulphide is formed. K. A. H. Mörner[1] of Stockholm, studied

(1) Zeit. für Physiol. Chemie, 34, 207 (1902).

this reaction quantitatively to determine what percentage of the total sulphur of keratine from different sources is split off to form lead sulphide. The following table gives the results of some of Mörner's researches:

Source of Keratine.	Total Sulphur.	Lead Blackening Sulphur.	Lead Black. S. / Total S.
Horn.	3.37%	2.48%	73.6%
Human hair.	5.26	4.16	79.1
Ovokeratin (Shell membrane of egg)	3.29	2.46	74.8
Feathers.	1.29	2.66	48.5

Material Used.	Tot. S.	Lead Blck. S.	Lead Black S. / Total S.
Crystalline egg Albumen	1.59%	0.43%	27.0%
Cystine	--	--	75.1%

Oskar Löw criticised Fischer's method, stating that the process of hydrolysis with boiling hydrochloric acid (sp. gr. 1.19), is too harsh a treatment of the keratine, for the strong acid employed completely destroys the identity of the original substance. If keratine could be broken down by milder reagents, perhaps a better clue to its true character could be gained, than is the case when drastic methods of attack are employed. With this latter thought in view, a search for new solvents for keratine was begun.

Experimental Part.

The new solvents for keratine which were found are as follows:

Phenylhydrazine (on boiling)
Monochloracetic acid.
Dichloracetic acid.
Trichloracetic acid.
Cyanacetic acid.
Glycerol.
Resorcinol.
Pyrogallol.
Pyrocatechol.
Hydroquinone.

In each case the pure substance was employed as solvent. Aqueous solutions of these substances are not suitable as solvents. Dichloracetic acid is the only solvent which acts at room temperature. There is no definite solubility of keratine in dichloracetic acid, the case being parallel to that of gelatine and water. The keratine slowly swells up if only a small amount of dichloracetic acid is added. As more acid is added keratine becomes softer and more jelly like. Upon further addition of acid the jelly becomes thinner and thinner until finally a solution is obtained. A bright purple to violet color develops which is destroyed upon heating on a water bath. Upon the addition of water to a solution of keratine in dichloracetic acid, a voluminous white, curdy precipitate is formed, which was found to be digestible with pepsin.

Monochloracetic and trichloracetic acids, being crystalline solids at room temperature, must be heated on the water bath to bring them into a liquid state. At the temperature at which these acids are in the liquid phase, (above 62° C.), they readily dissolve keratine, in these cases also with the formation of a purple color, which, however, is soon destroyed upon continued heating.

Cyanacetic acid, too, at 90° readily dissolves keratine. Upon the addition of water to such a solution, a dirty grey precipitate is obtained.

Resorcinol, pyrogallol, pyrocatechol, and hydroquinone have solvent action when fused to the liquid phase at temperatures from 165° to 250° C. When these compounds react with keratine, hydrogen sulphide is liberated. Keratine is also slowly dissolved upon heating with glycerol. At about 200°, hydrogen sulphide begins to come off.

What part of the total sulphur is evolved as hydrogen sulphide, and whether this sulphur bears any relation to the "lead blackening sulphur" investigated by K. A. H. Mörner, seemed an interesting problem for investigation. The materials used in this investigation were horse hair, human hair, cartilage from a sturgeon's snout, and keratine from the inner white part of the horse's hoof. In each case the finely divided material was well washed, treated with five per cent hydrochloric acid, and then finally extracted with alcohol and ether.

In the case of the hoof keratine, the material was also treated with pepsin-hydrochloric acid digestive solution[1] and then with tryptic digestive solution.[2] The material was finally dried at 100° in an air oven. The total sulphur of each of these substances was determined by means of sodium peroxide fusion in nickel crucibles.[3]

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[1] The solution had the following composition:

Hydrochloric acid - - - 0.25%

Pepsin - - - - - - - - - 0.10%

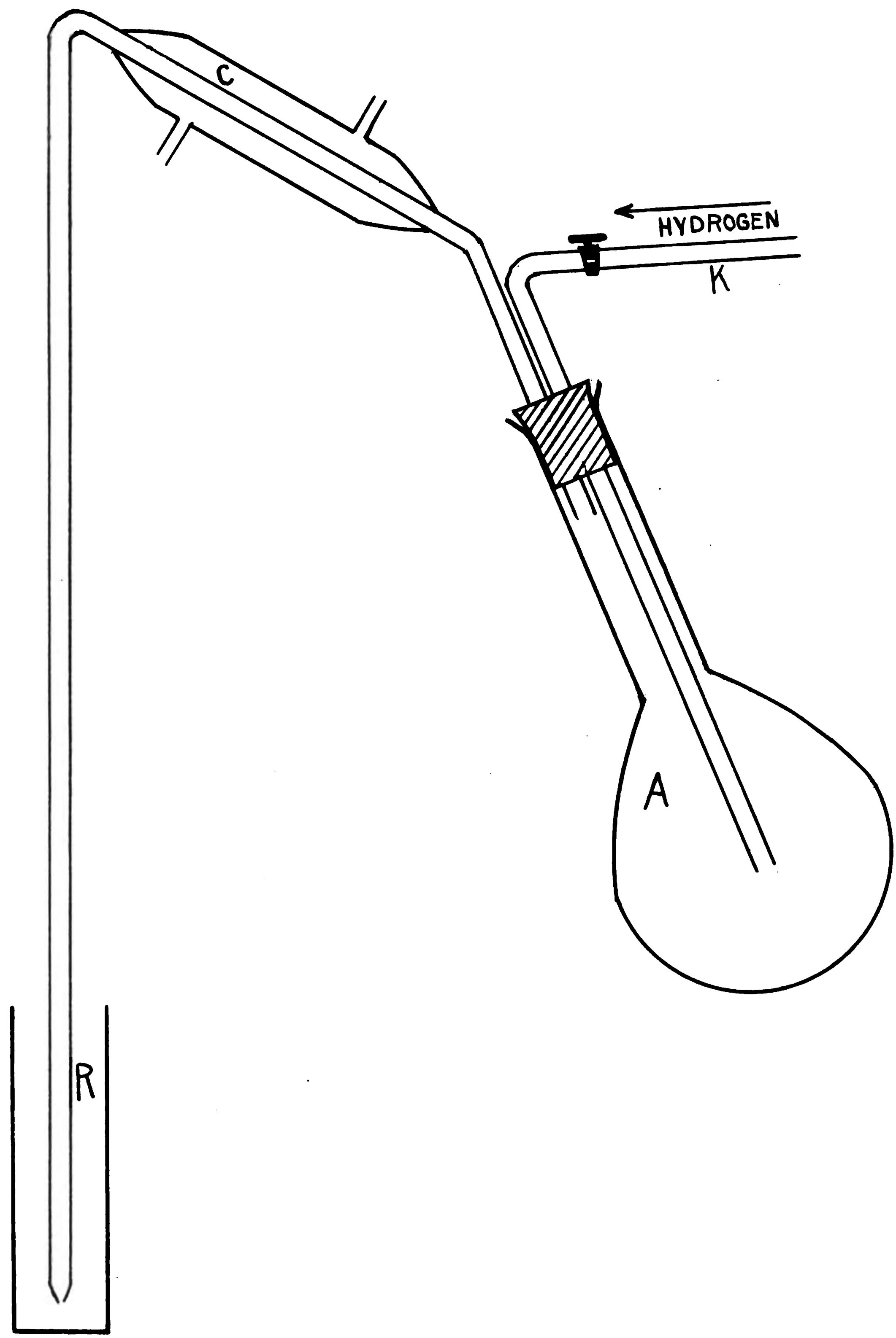
[2] The tryptic digestive solution was made as follows:

Trypsin powder - - - - 0.25%

Sodium carbonate - - - 1.00%

[3] Dept. of Agric. Bulletin No. 107 - "Official and Provisional Methods of Analysis."

To determine the amount of sulphur given off as hydrogen sulphide when keratine and other proteins are dissolved in glycerol, the apparatus in the accompanying sketch was used.


C
HYDROGEN
K
A
R

The sample, together with 60 gm. of glycerol, was placed in the 250 cc. Kjeldahl flask A. Hydrogen was then passed through the apparatus for fifteen to twenty minutes until all the air was surely displaced. A low flame was applied to the Kjeldahl flask, and in a few minutes the glycerol seemed to boil, due to the evolution of hydrogen sulphide. Solution of the keratine took place simultaneously. The hydrogen sulphide was passed over into the receiver R., which contained an ammoniacal solution of five per cent hydrogen peroxide. The condenser C prevented any liquid from passing over into the receiver R. The hydrogen sulphide, of course, was oxidized to ammonium sulphate by the hydrogen peroxide. The sulphur was finally precipitated and determined as barium sulphate.

The following table gives a summary of the results obtained. The sulphur evolved as hydrogen sulphide by this reaction with glycerol, is termed as "glycerol sulphur."

Material	% Total Sulphur.	Wt. of sample used to det. glycerol S.	Wt. of Ba SO_4 obtained	% Glycerol Sulphur	Glycerol S / Total S. in %
Horse's hoof	2.31	0.3076	0.0238	1.06	
		1.0125	0.0812	1.10	
	2.32	1.2251	0.1050	1.18	
	2.23	1.0905	0.0969	1.22	
		0,8334	0.0594	1.24	
		1.2354	0.1124	1.25	
		0.3094	0.0282	1.25	
		0.3004	0.0277	1.26	
		0.3006	0.0331	1.51	
Average	2.29			1.23	53.7
Human hair (brown)	5.14	0.6684	0.1274	2.62) a	
	5.17	0.6765	0.1314	2.67) a	
	5.43				
		0.6670	0.1290	2.66) b	
		0.6786	0.1344	2.72) b	
Average	5.25			2.67	50.7
Horse hair (black)	4.05	0.5720	0.0604	1.45	
	4.06	0.6814	0.0724	1.46	
		0.7126	0.0819	1.58	
Average	4.06			1.50	37.00
Cartilage from sturgeon's snout	2.18	0.8108	None	None	
	2.24	0.8016	"	"	None
		0.3990	"	"	
		0.4034	"	"	

It is quite remarkable that the cartilage from the sturgeon's head gave off no sulphur as hydrogen sulphide. Gelatine, too, it was found gives off none of its sulphur as hydrogen sulphide when treated with hot glycerol. From the table it will be seen that the keratine from hoofs evolved the highest amount of sulphur as hydrogen sulphide. In the case of human hair, the percentage of the total sulphur evolved as hydrogen sulphide is lower, and horse hair yielded a still lower percentage.

The method of determination used would, however, allow the escape of sulphur if the latter were evolved in the form of mercaptans or disulphides. It was thought that possibly an appreciable amount of sulphur might escape in such organic compounds. To determine whether this is actually the case, the hydrogen sulphide, and any other gases evolved, were passed through a red-hot Jena tube (about two feet long), with a current of hydrogen. Any mercaptans or disulphides present would by this method be converted into hydrogen sulphide, and the corresponding hydrocarbon. The hydrogen sulphide could then be absorbed as before. In the case of human hair, this additional precaution of passing the gases through a red-hot tube was taken, but the results showed that the amount of sulphur present as mercaptans or disulphides, is less than the experimental error.

In the previous table the results marked (a) were obtained without the use of a red-hot tube; those marked (b) were obtained with the use of a red-hot tube. It may readily

be seen that the difference is not appreciable. However, when the determination was made without the use of a red-hot tube, an exceedingly disagreeable odor was noticeable, whereas when a hot-tube was used absolutely no odor at all was produced, showing that all mercaptans or disulphides were destroyed,- that is to say converted into hydrogen sulphide and then absorbed by the absorbing agent. The amount of sulphur present, however, in these ill smelling compounds as appears from these determinations, is very minute.

SUMMARY.

Although keratine is extremely resistant material, a number of new solvents were found, among these are:-

Cyanacetic acid.

Monochloracetic acid.

Dichloracetic acid.

Trichloracetic acid.

Phenylhydrazine.

Resorcinol.

Glycerol.

Pyrogallol.

Pyrocatechol.

Hydroquinone.

The pure substances act as solvents,- aqueous solutions do not do so. In many cases dilution with water will cause the precipitation of the dissolved keratine. The precipitate formed upon diluting a solution of keratine in dichloracetic acid was found to be digestible with pepsin. The amounts of sulphur evolved as hydrogen sulphide by the action of hot glycerol upon various forms of keratine and other forms of protein, varies considerably, keratine from hoofs yielding the highest percentage of hydrogen sulphide.

The cartilage found in the sturgeon head, although containing 2.2% sulphur, yields no hydrogen sulphide at all upon boiling with glycerol. Gelatine behaves similarly. Thus far no relation is apparent between Mörner's values of "lead blackening sulphur", and the values here obtained for glycerol sulphur.

This work was done at the suggestion of and under the direction of Professor Louis Kahlenberg.

Approved L. Kahlenberg

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